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Research Opportunities for Flight Experiments in Space Life Sciences

Title: Bioavailability and Pharmacodynamics of Promethazine on Long
Duration Missions to the International Space Station

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III. Abstract. Space motion sickness (SMS) is often treated in space with promethazine (PMZ). Common side effects of PMZ administration (50 mg intramuscular) on the ground are drowsiness and impaired cognitive performance. Anecdotal reports indicate that these effects are absent or less pronounced in space. This suggests that the availability of PMZ to the body (bioavailability) and/or the response of the body to PMZ (pharmacodynamics) may change during space flight. Opportunities for clinical research in space are limited. The study described here is our response to a NASA Research Announcement for proposals for flight-based research needed to improve, or answer specific questions about, diagnosis and therapy during space flight, and post-flight rehabilitation.

We propose here to evaluate noninvasive methods for determining the bioavailability and pharmacodynamics of PMZ. The specific objectives of the proposed research are to 1) compare pharmacokinetic and pharmacodynamic parameters of PMZ, estimated from saliva and plasma levels after administration of PMZ, 2) estimate the relative bioavailability of the three dosage forms of PMZ that are often administered to control motion sickness symptoms in space, and 3) establish the dose-response relationship of PMZ.

We will estimate the bioavailability of an intramuscular injection (IM), oral tablet, and rectal suppository of PMZ in normal subjects during ambulatory and antiorthostatic bed rest (ABR) conditions using novel stable isotope techniques. We will compare and contrast the bioavailability of PMZ during normal and microgravity conditions to examine changes in drug absorption and bioavailability during microgravity. Results of this study will validate methods for an approved in-flight investigation with this medication awaiting an opportunity for manifestation..

IV. Specific Aims. These are the working hypotheses of this project:

- A. Relative bioavailability of PMZ from different routes of administration can be estimated by simultaneous administration of three different stable isotopic forms of PMZ.
- B. A consistent ratio between saliva and plasma concentrations of PMZ exists following all routes of administration of PMZ. This ratio may be altered during spaceflight.
- C. Spaceflight will induce changes in the absorption, bioavailability, and pharmacodynamics of PMZ.

The overall goal of the proposed research is to characterize the pharmacotherapeutic profile of PMZ in spaceflight. The study will be conducted with 12 astronauts or cosmonauts, with an equal number of each gender. These are the specific aims designed to achieve the research goal:

- Specific Aim 1. During ground and spaceflight conditions and using stable isotope formulations, estimate the bioavailability of PMZ after administration by intramuscular, oral, and rectal routes.
- Specific Aim 2. After collecting saliva and blood samples, determine the saliva-to-blood plasma ratio of PMZ. Validate the feasibility and reliability of using saliva levels of PMZ as a noninvasive method to study the pharmacokinetics and pharmacodynamics of PMZ in space.
- Specific Aim 3. Examine the effect of spaceflight on the absorption, bioavailability, and pharmacodynamics of PMZ.
- Specific Aim 4. Establish noninvasive methods for determining the pharmacodynamics of PM. These methods may include collecting saliva samples, administering cognitive performance tests, and using activity monitoring devices.

V. Background and Significance. The biological effect elicited by a therapeutic agent in a given dosage form, under any condition, is a function of the agent's intrinsic activity and its concentration at the site of action. The onset, intensity, and duration of the pharmacological response produced by a drug, collectively known as that drug's pharmacodynamics, depend upon the processes that affect its concentration, namely, absorption, distribution, metabolism, and elimination. The mathematical description of these processes and their interrelationships are known as that drug's pharmacokinetics. In general, understanding the kinetics and dynamics of a drug allows the development of effective dosage regimens; allows appropriate adjustments for variations among individuals; and allows appropriate corrective actions in the event of an unusual response to that drug. Both pharmacokinetic and dependent pharmacodynamic parameters (e.g., bioavailability, clearance, and onset, magnitude, and duration of effect) are influenced by many physicochemical, biological, and clinical factors. These factors include but are not limited to subject's gastrointestinal, hepatic, and renal function, body weight and size, disease state, as well as a drug's protein-binding characteristics, and its interaction with other drugs administered simultaneously.

Exposure to microgravity causes complex physiological changes in humans. The degree and magnitude of these changes probably vary with mission length and other mission-related factors. These physiological changes in turn induce therapeutically significant changes in the pharmacology of drugs used during space flight. Changes in gastrointestinal function, redistribution of body fluids, and changes in hepatic and renal function are known to affect drug absorption, distribution, metabolism, and elimination (Bennett et al., 1978; Klotz, 1976; Levine, 1970; Wilkinson, 1976; Williams and Namelok, 1980). Since orbital space flight is known or suspected to affect all of these systems, it is very important to characterize the induced changes and associated pharmacological consequences in order to develop effective treatments for in-flight pathologic incidents.

Pharmacokinetics in space. Conventional methods of collecting pharmacokinetic information require multiple blood samples to determine drug concentrations in the body over time. As an alternative to this invasive procedure, which is also difficult to perform in microgravity, saliva was found to be useful for estimating drug bioavailability of many drugs like acetaminophen and scopolamine. The validity of using drug concentrations in saliva to predict blood levels depends on both the concentration of a drug that appears in saliva and the consistency of its saliva-to-plasma (S/P) ratio over a range of plasma concentrations (Graham, 1982). Many weakly basic drugs distribute readily into saliva at measurable concentrations (Danhof and Breimer, 1978). Salivary concentrations of highly protein-bound drugs represent the active (unbound) form of the drug, while plasma concentrations represent both active and bound fractions (Vesell et al., 1975).

Limited results from ground-based simulation studies and preliminary flight studies using saliva to monitor acetaminophen and scopolamine suggest that microgravity induces changes in the absorption of drugs after oral administration (Cintrón et al., 1987; Putcha et al., 1989; Putcha and Cintrón, 1991). Since absorption is directly related to bioavailability, a change in absorption can potentially affect a drug's anticipated therapeutic effect by altering the rate of onset, the magnitude, or the duration of that effect. It is suspected that changes in GI function associated with microgravity are at least partially responsible for altering absorption patterns. Because GI function directly influences the absorption of nutrients and fluids as well as drugs, alterations in GI function will become more important as space missions become longer.

Ground-based analogs for microgravity. Flight opportunities to conduct space research are limited and require prolonged periods of preparation before implementation. Therefore, researchers have used ground-based microgravity analogs to simulate physiological and biochemical changes similar to those observed during space flight. The most widely used simulation model of space flight environment for human research is bed rest, both horizontal and head-down (antiorthostatic) posture (Nicogossian et al., 1979). Bed rest has been reported to induce many physiologic changes that are similar to those observed in space such as muscle atrophy, bone demineralization, redistribution of fluids and body mass, and decreases in plasma volume and red blood cell mass (Genin, 1977; Sandler, 1976; Sandler and Vernikos, 1986). On the other hand, antiorthostatic bed rest (ABR) elicits some of the early physiological effects of microgravity more precisely than the horizontal bed rest (Kakurin et al., 1976). In particular, ABR results in more rapid and pronounced fluid shifts and resultant cardiovascular changes (Blomquist et al., 1980).

In a study on the effects of ABR on physiology and metabolism in human subjects, ABR has been shown to induce changes in gastrointestinal (GI) motility similar to those observed in a limited number of crewmembers during space flight. (Putcha, 1991; Lane, et al., 1999). A physiologically based pharmacokinetic model for acetaminophen was constructed using data from this study and similar data collected during space flight were fitted to this model. A high degree of correlation was noticed between these data indicating that ABR may be a good surrogate for microgravity (Srinivasan et al., 1994).

Since absorption and bioavailability of oral medications are dependent on GI motility, and for drugs that have relatively low bioavailability from an oral dose like scopolamine and PMZ, ABR may serve as an effective simulation model to examine bioavailability changes of these drugs during space flight. Another fact that supports the use of ABR for bioavailability evaluations of SMS medications is that it induces early physiological effects of microgravity, and SMS is manifested during early flight days.

Pharmacology of space motion sickness. Approximately 70% of Space Shuttle crewmembers experience symptoms of SMS during the first few days of flight. These symptoms can include malaise, anorexia, headache, lack of motivation, impaired concentration, stomach awareness, and vomiting (Reschke et al., 1994). The frequency and severity of SMS symptoms has led to restrictions on extravehicular activities (no sooner than 72 hours after launch) and mission duration (no less than 3 days).

Tests on Earth suggest that motion sickness is associated with a profound reduction in gastric emptying (Stewart et al., 1993; Wood et al., 1987). Although the specific mechanisms remain unknown, a reduction in gastric emptying typically has two effects on orally administered drugs: a delay in the time of peak concentration and a reduction in the peak concentration of drug in blood (Nimmo, 1981). These changes affect the rate and extent of a drug's pharmacodynamic effect, which may partially explain why some orally administered drugs seem to be less effective in space. However, many other factors typical of space flight, such as cephalad fluid shifts, cardiovascular changes, changes in cabin pressure, and dehydration, will influence drug absorption and metabolism from any route of administration (Santy and Bungo, 1991).

Numerous medications have been used, with varying success, to treat SMS symptoms. Currently, PMZ is the antimotion sickness drug of choice for Space Shuttle missions, and has a reported 75% efficacy via the intramuscular route (Davis et al., 1993). PMZ, a phenothiazine and an H-1 antihistamine, is used before and after surgery as an adjunct to analgesia and for its sedative and antihistaminic effects. PMZ can be given intravenously (IV), orally (PO), intramuscularly (IM), or as a suppository. PMZ's moderate anticholinergic effects are thought to be the source of its antimotion sickness activity (Wood and Graybiel, 1972). Typical side effects include dizziness, drowsiness, sedation and impaired psychomotor performance (all typical of anticholinergics); extrapyramidal and dystonic reactions are typical of phenothiazines and other dopaminergic receptor antagonists.

Pharmacokinetics and bioavailability of PMZ. The pharmacokinetics of PMZ have been characterized after IV, PO, and IM doses. After IV doses, plasma levels decline exponentially with a terminal elimination half-life of 7 to 14 hours (Taylor et al., 1983). Absolute bioavailability after an oral dose is low, averaging approximately 22-25 percent, with plasma concentrations peaking 6 to 12 hours after PO or IM doses; peak plasma concentrations after IM administration are about 4 times those after an equivalent PO dose. PMZ's low bioavailability after oral administration may be due to significant first pass metabolism and/or poor absorption from the gut. PMZ has a large volume of distribution (970 L) and is about 80 percent protein bound. It is cleared from the body by

hepatic metabolism; promethazine sulfoxide is the major metabolite and desmethylpromethazine is a minor metabolite. Less than one percent of the drug is eliminated unchanged in the urine. The saliva (parotid) to whole blood ratio is about 0.24 after IM and 0.20 after PO administration (DiGregorio and Ruch, 1980, Taylor et al., 1983).

Novel clinical research technologies. Conventional pharmacokinetics and bioavailability studies described above are conducted using extensive crossover study designs with multiple treatment sessions that prolong the experimental period and often compromise subject compliance. Such crossover studies are based on the assumption that a drug's clearance is constant over long periods of time and have minimal intra-individual variability throughout the study. Unfortunately, these studies also require a large number of subjects for long time periods in order to minimize subject variability and maximize reliability of results. Studies with drugs where systemic clearance is strongly influenced by factors such as posture, GI function, or blood flow changes to the eliminating organ, the assumption of constant clearance may no longer be valid and a simple crossover design is neither appropriate nor practical (Powell, 1997). Additionally, because hepatic metabolism is characterized by high inter- and intra-individual variability, it is often difficult to extrapolate clearance either between two doses in the same individual or two doses in two individuals (Lesko et al., 2000). Further, these study designs may be also inappropriate for similar investigations in space flight due to the anticipated dynamic organ function and blood flow changes induced by the microgravity environment.

This simultaneous administration of SIL and unlabeled PMZ protocol has been selected because of reports using similar protocols (Gilbert, et al., 1998; Browne, et al., 1993) for bioavailability and bioequivalency studies. These investigators used an unlabeled drug with two labeled drug forms (timolol and phenytoin) in their studies.

The advent of LC-MS technology has led to the development of novel approaches using stable isotope labeled (SIL) drug analogs in clinical biomedical research. Mass spectrometry (MS) has been the premier technique for the detection, structure determination and quantitation of compounds labeled with stable isotopes. The on-line combined technique of liquid chromatography-mass spectrometry (LC-MS) provides the advantages of both high performance liquid chromatography (HPLC) and mass spectrometry. LC-MS is widely recognized as the most powerful tool available for the analysis of low concentrations of drugs and their metabolites in biological matrices, with high sensitivity and specificity. The ability of LC-MS to distinguish and measure compounds (drugs & metabolites) labeled with stable isotopes with such analytical prowess, makes this technique the obvious choice when tracer studies with pharmaceuticals are considered (Evans, 1997). As a result, SIL formulations have been successfully employed by the pharmaceutical industry and by clinical researchers for the assessment of absolute and relative bioavailability of drugs (Gilbert, et al., 1998; Powell, 1997). SIL forms commonly used for clinical pharmacokinetic studies are deuterium, ^{13}C , ^{15}N and ^{18}O . Careful selection of the type and location of isotope label, and simultaneous administration of the labeled and unlabeled drug forms will avoid metabolic isotope effect and other related isotope effects on pharmacokinetics and bioavailability measurements.

Study designs utilizing SIL techniques offer greater statistical power and less intra-individual variability for bioavailability assessments of drugs (Gilbert, et al., 1998; Powell, 1997; Browne, et al., 1998). The most important advantage of these techniques is subject safety enhanced by the absence of ionizing radiation doses, and reduction in sample requirements since multiple formulations of a drug in different SIL forms can be administered simultaneously and circulating concentrations measured in a single sample using LC-MS techniques (Klein, 1997). These studies also offer higher statistical power for detecting changes since inter-subject variability between treatments is eliminated by simultaneous administration of test doses.

Pharmacodynamics and side effects of PMZ. Surprisingly, there is very limited information available on the pharmacodynamics of PMZ, while there are a number of reports on the side effects of the medication. Although any side effect can be problematic in space, none can endanger crew safety more than the impairment in psychomotor performance. Space crews often must perform complex, exacting tasks during the first few days in space, when SMS symptoms are worst. Crews' cognitive, motor, and perceptual skills must be preserved in space. Oral doses of PMZ (12.5 to 25 mg) on Earth are known to impair psychomotor performance, information processing, and feelings of alertness, with deficits peaking within 3 to 4 hours after dosing and that lasted for as long as 8 to 9 hours (Parrott and Wesnes, 1987). PMZ also impairs performance on operationally structured tests (Hyman et al., 1988; Schroeder et al., 1985). In a recent study on the effects of PMZ (30 mg PO), cognitive and psychomotor function in normal subjects was impaired as indicated by a significant reduction in critical flicker fusion threshold and an increase in recognition reaction time and percent day time-sleep like activity (Hindmarch et al. 1999).

Anecdotal reports of PMZ users during flight suggest that the central nervous system (CNS) effects of this drug may be attenuated in space (Bagian, 1991; Davis et al., 1993). Objective assessment of performance-related effects, in combination with bioavailability, will promote the establishment of safe and effective doses for PMZ in space. We, therefore, propose a systematic evaluation of the bioavailability and pharmacokinetics of PMZ in conjunction with an assessment of the drug's pharmacodynamic effects, especially the performance decrements, in astronauts on long duration missions to the International Space Station.

VI. Preliminary Studies. We evaluated in-flight use of medications from astronaut debriefings after 79 U.S. Space Shuttle missions (Putcha, et al., 1999). From the 219 records obtained (each representing one person-flight), 94% included some medication being taken during flight; of that number, 47% were for space motion sickness, 45% for sleep disturbances, and smaller percentages for headache, backache, and sinus congestion. Drugs were given most often orally, followed in decreasing order of frequency by intranasal, intramuscular, and rectal routes. Promethazine (PMZ) is the only drug that is given by three different routes, oral, intramuscular, and rectal during space flight. Since motion sickness has been one of the major discomforts during short duration space flights, and scopolamine and PMZ are identified as the drugs of choice for the treatment of this ailment, we examined the pharmacokinetics of these two drugs. Antiorthostatic bed rest

(ABR) has been used for validating noninvasive methods and for evaluating some of the physiological and pharmacokinetic changes using these noninvasive methods. Results from some of these studies are presented here.

Scopolamine. Scopolamine is used to alleviate SMS symptoms in space. The drug has poor and variable oral bioavailability (Putcha et al., 1989). We evaluated the pharmacokinetics of orally (PO) and intravenously (IV) administered scopolamine after 24 h of ABR to examine the drug's oral bioavailability in a simulated microgravity environment. Pharmacokinetic analysis indicated significant decreases in the absorption and bioavailability of oral scopolamine (Putcha et al., 1989); distribution and elimination of intravenous scopolamine were no different during ABR than in the controls. These results were in agreement with results from a similar investigation during space flight. During ABR and during space flight, absorption and bioavailability of scopolamine were decreased; whereas, distribution and elimination of the drug were not different from the respective controls (Cintrón et al., 1987). These data suggest that ABR, even for a short duration of 24 h can simulate changes in drug bioavailability similar to the ones observed during space flight.

Based on these results, we examined the effect of ABR on gastrointestinal function, a key physiological factor of absorption and bioavailability of orally administered drugs. We validated a noninvasive breath hydrogen test in conjunction with acetaminophen absorption test to estimate changes in gastrointestinal (GI) motility and absorption during ABR and during space flight. Results from these studies indicate that GI motility during ABR and during short and long duration space flights was reduced significantly and ABR simulated changes in the GI motility and absorption similar to the ones noticed during limited space flight experiments (Putcha, 1991; Lane et al., 1999). A physiologically based pharmacokinetic model for acetaminophen was constructed using data from the ABR study and the model was fit to data collected during space flight. A strong correlation was noticed between model-predicted and space flight data indicating that ABR may be a good surrogate for microgravity for absorption and bioavailability studies.

Promethazine. In a recent pilot study on methods validation in support of an approved flight investigation, we evaluated the pharmacokinetics and pharmacodynamic effects of a single intramuscular administration of PMZ (50 mg) in commercial airline pilots trained to operate a space shuttle landing simulator. A single dose of 50 mg induced a statistically significant increase in perceived drowsiness at 1, 4, and 8 hours after administration compared to placebo controls. Similarly, Stanford Sleep Scale Scores were significantly higher at 1, 2 and 4 hours after PMZ administration compared to placebo controls. These results suggest that a 50 mg intramuscular dose of PMZ can induce drowsiness in pilots that lasted up to at least 4 h after administration.

For estimating metabolite levels, an on-line solid phase extraction (SPE) liquid chromatographic (LC) method using column-switching system has been developed and utilized for the simultaneous measurement of PMZ and its metabolites. The method employs direct injection of human urine into an on-line SPE column for the effective removal of matrix interference, which allows the enrichment of the analytes. The use of

on-line SPE resulted in reduced sample preparation time, cleaner extract and greater reproducibility. The linear dynamic range covered was from 1 to 1200 ng/ml with a correlation coefficient of 0.999 (using 1/X as a weighting factor). The limit of detection (LOD) of this assay using UV detection varied from 4.25 to 5.39 ng/ml. And the sensitivity was enhanced to the picogram level using fluorescence detection with an excitation wavelength at 254nm and emission wavelength 425nm (LOD 285 pg/ml for PMZ and 248 pg/ml for desmethyl PMZ). Quantitative data demonstrate that the assay is specific, precise, accurate, and robust with a wide concentration range. A publication describing the new method has been accepted for publication in the *J of Chromatography*.

We also examined in this study, the saliva to plasma ratio of the parent compound (PMZ) and a major metabolite (PMZ sulfoxide). As seen in the representative profiles of saliva and plasma in one subject, a consistent ratio between saliva and plasma exists for both the compounds, however, the ratio is approximately 0.8 for PMZ sulfoxide while it is about

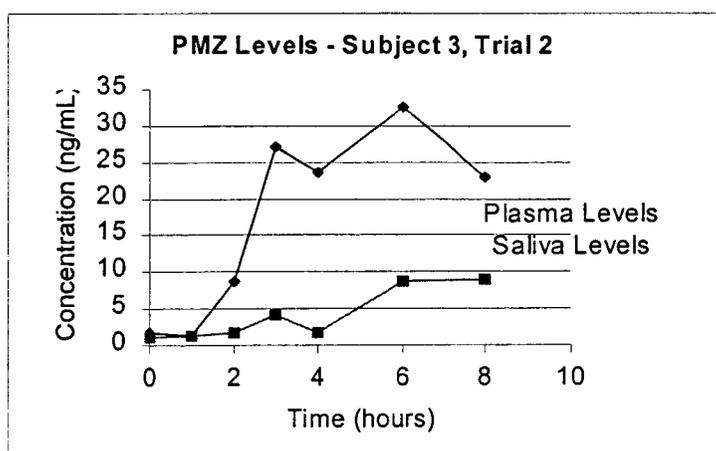


Figure 1A: Plasma and saliva levels of PMZ; determined by HPLC with UV detection.

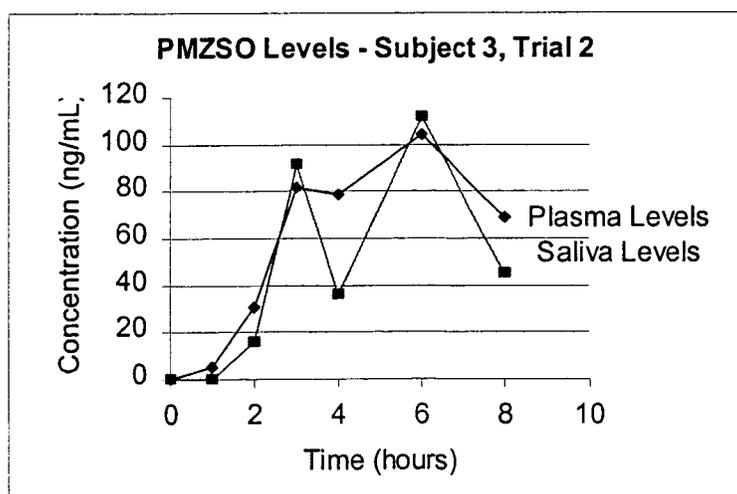


Figure 1B: Plasma and saliva levels of PMZ sulfoxide; determined by HPLC with UV detection.

0.4 for PMZ (Figures 1A & B). Results from this study, however, will not provide information regarding changes in this ratio that may be anticipated in a microgravity environment.

In preparation for the proposed research, we procured one SIL PMZ +4 form, deuterated at the 1, 3, 7, and 9 position on the aromatic rings (C/D/N Isotopes, Point-Claire, Quebec, Canada) for the development and validation of an LC/MS assay to quantitate PMZ and +4 PMZ. As shown in Figures 2, selective ion monitoring of authentic standards of PMZ, deuterated PMZ, and trifluoperazine (internal standard) is used to quantitate PMZ levels. The linear dynamic range covered with this assay is from 5 to 300 ng/ml (Figure 3). This assay, in conjunction with the Waters Alliance/ Micromass Z-spray LC-MS, will be employed for the simultaneous determination of unlabeled PMZ, SIL +4 PMZ and SIL +7 PMZ, and their respective labeled and unlabeled metabolites in the Phase I bioavailability study.

In a separate, ongoing study in our lab investigating the bioavailability and pharmacodynamics of PMZ in human subjects in both ambulatory and ABR scenarios, PMZ was found to significantly affect several aspects of cognitive performance, as measured by the ARES and WinSCAT cognitive performance software tools. Time to accurately complete memory tasks was found to increase significantly with concentrations. Higher concentrations also increased response time and decreased accuracy of substitution and matching tasks. AUC and half-life estimates for PMZ ranged between 0.12 and 1.7 mg.h/L and 15 and 50 h, respectively.

The simultaneous administration of SIL formulations of PMZ in this study have shown that oral administration in an ambulatory setting provides the longest exposure time to PMZ, likely because much of the PMZ is immediately metabolized to conjugates which are then excreted into the gut, metabolized there by bacterial flora or intestinal cytochromes back to the parent compound, PMZ, and then reabsorbed into the circulation in a process called enterohepatic recycling. Because of this recycling, peak concentrations may occur more than 24 hours after the initial dose in subjects who have a high propensity to excrete via conjugation, such as females and certain ethnic groups. During ABR however, intramuscular administration (to the buttocks) produced the highest bioavailability, 26 and 13 times higher than rectal suppository or oral capsule. Finally, PMZ may exhibit dose-dependent pharmacokinetics in humans as AUC increased disproportionately with dose, a common feature of drugs excreted by conjugation.

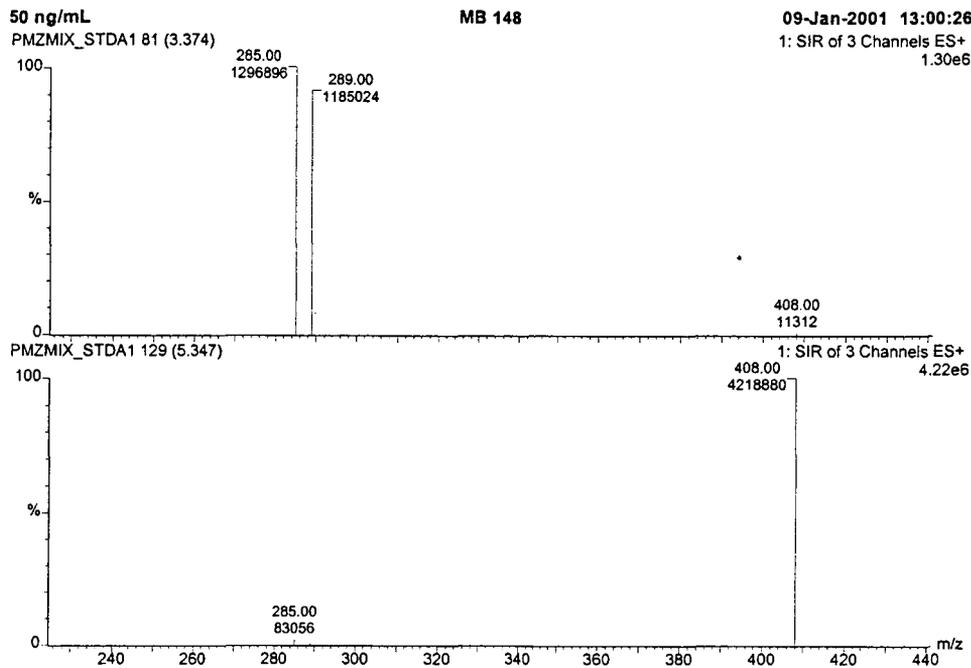
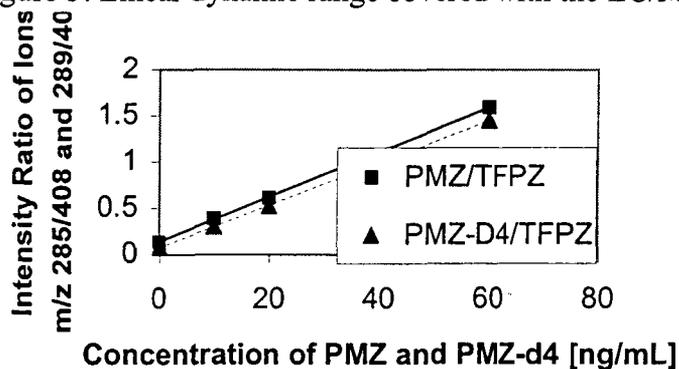


Figure 2: Mass spectra of PMZ (m/z 285), +4 PMZ (m/z 289), and an Internal Standard trifluoperazine (TFPZ), (m/z 408).

Figure 3: Linear dynamic range covered with the LC/MS assay.



Sleep and activity measurements. We have used and validated in a related investigation, the methods we propose to collect sleep and performance parameters in this study. We examined the sleep quality by measuring sleep-related parameters in astronauts working on altered work-sleep schedules aboard the space shuttle. Sleep parameters were measured using wrist actigraphy before flight, during preflight light treatment period, and after flight. The data generated by the actigraph were analyzed for the sleep-wake cycle, the quality of rest (number of minutes awake after sleep onset), and sleep to wake ratio (Putcha, et al., 1997). Results of this study suggested that actigraphy could successfully detect changes in sleep intensity and quality. Based on this experience, and on a recent report that actigraphy is a sensitive measure for assessing drug induced changes in percent daytime activity and sleep (Stanley, 1997), we plan to use these methods in the proposed investigation to collect data on the parameters described above.

Cognitive performance measurement tool. The WinSCAT (Windows based Shuttle Cognitive Activity Test) was developed exclusively for space flight-specific cognitive activity testing. WinSCAT has been used by astronauts during the NASA-7 mission to Space Station Mir and during the Lunar/Mars Life Support Test Project (LMSTP) to validate sensitivity and applicability to shuttle operational testing. A study to validate the WinSCAT against widely used neurocognitive measures (Letter-number Sequence, Digit Symbol, Digit Symbol Incidental Memory, Figural Memory, Paired Associate Learning, Trails B, and Paced Serial Addition Test) was conducted in young adults. Results of this study indicated that there is a significant relationship between the WinSCAT tests and the widely used tests of neuropsychological function. Another study was conducted to assess the sensitivity of WinSCAT to detect hypoxia effects on performance, results indicated a significant difference in WinSCAT scores with subjects breathing hypoxic air equal to 15,000 feet mean sea level and normoxic air. However, in a study with subjects completing a military survival course indicated that WinSCAT scores were not sensitive to high fatigue as measured by other fatigue measurements. A study to examine the learning effects and score stability using WinSCAT in 60 medical students over a one-year period is in progress at this time. We are encouraged by the applicability of the WinSCAT for detecting neurocognitive effects of these above mentioned off-nominal conditions and are proposing to use the test for pharmacodynamic evaluation of PMZ.

VII. Research Design and Methods. This research protocol is designed in response to the NASA Research Announcement charge for flight experiments to investigate critical aspects of biology and medicine in spaceflight, in preparation for upcoming missions to the Moon and Mars. The proposed protocol utilizes novel methods for the assessment of pharmacodynamics and estimation of bioavailability changes during space flight. Further, it is aimed at developing and validating noninvasive methods to characterize pharmacodynamic effects of neurosensory medications in general, and PMZ in particular.

a. Study design overview. The protocol is designed to estimate the bioavailability of PMZ using novel stable isotope label (SIL) techniques that minimize subject experimentation time and exposure to risk. This study is designed so that intra-subject variability is minimized, thereby improving estimates of relative bioavailability of multiple dosage forms in individual subjects. A total of 12 astronauts will participate in the study.

The study consists of four sessions: 1) pre-flight, 2) FD3, 3) FD10, and 4) FD120. Each session consists of 72 h of sample and data collection.

Each subject will receive, simultaneously, an intramuscular (IM) injection (12.5 mg) of unlabeled PMZ, an oral (PO) capsule (12.5 mg) of stable isotope labeled (SIL) PMZ (+4) and a rectal suppository (12.5 mg) of SIL PMZ (+7), once during each session. The PMZ doses were selected so that the combined bolus dose does not exceed the maximum recommended therapeutic dose of 50 mg.

Baseline Data Collection: Session one is for baseline data collection. Pharmacokinetics of promethazine will be determined on the ground at a time before mission preparation and be used as a comparison for early flight and later flight days.

Table 1: Experimental Design for Phase and Treatment Levels

Subject #	Treatment	Treatment	Treatment	Treatment
1	T0	T1	T2	T3
2	T1	T0	T3	T2
3	T2	T3	T0	T1
4	T3	T2	T1	T0
5	T0	T2	T3	T1
6	T2	T0	T1	T3
7	T3	T1	T0	T2
8	T1	T3	T2	T0
9	T0	T3	T1	T2
10	T3	T0	T2	T1
11	T1	T2	T0	T3
12	T2	T1	T3	T0

T0: placebo; T1: 12.5 mg PMZ; T2: 25 mg PMZ; T3: 50 mg PMZ

b. Subjects. Twelve healthy, non-smoking astronauts between the ages of 21 and 45 years will participate in the study. All subjects will be screened and selected to the inclusion and exclusion criteria listed below.

Inclusion criteria:

1. Male or female
2. Age 21-45
3. Normal weight for body size
4. General good health, as determined by an Air Force Class III Flight Physical
5. Renal and hepatic function within normal ranges
6. Able to provide written informed consent to participate

Exclusion criteria:

1. Drug allergies, especially to phenothiazine-type agents
2. Use of prescription or over-the-counter medications within 3 days of starting the study
3. Use of an investigational drug within 30 days of starting the study
4. Tobacco smoking within the past year
5. Blood donation or significant blood loss within 30 days of starting the study
6. Significant gastrointestinal disorder, asthma, or seizure disorders
7. History of alcohol or other drug abuse
8. Pregnancy or suspected pregnancy, or lactation
9. Treatment during menstruation will be avoided
10. Hematocrit values less than 41% for males and 37% for females

c. Procedures.

Promethazine Dosage Forms. Two deuterated forms of PMZ (Figure 5) will be manufactured and supplied by ISOTEC Inc. (Miamisburg, OH) along with appropriate USP purity and isotope enrichment data. Capsules (opaque #6) will be formulated using +4 PMZ (12.5 mg) and dextrose; suppositories will be formulated using +7 PMZ (12.5 mg) with standard ingredients for suppository formulation and according to standard practice in compounding. IM PMZ will be purchased from Wyeth-Ayerst (Philadelphia, PA). Ten dosages from each formulation will be analyzed for verification of PMZ content. The oral and suppository formulations will be prepared under regulatory guidelines in an accredited pharmacy by a pharmacist licensed in the state of Texas using GMP guidelines. An IND approval for the SIL dosage forms from the FDA is not necessary before implementation of this study. Since the dosing routes proposed in this study are FDA approved for PMZ administration and since the combined dose of administration is within the therapeutic range, no difference is expected in the pharmacologic and toxicologic profiles of PMZ and the two deuterated forms.

Deuterium substitution at the specific labeling sites for the +4 and +7 deuterated isotopes of PMZ was chosen for the following reasons: deuterium is safe, inexpensive compared to other stable isotopes (^{13}C , ^{15}N), and the isotopes are easy to synthesize. The most important reason, however, is to avoid metabolic isotope effects. The propylamine side chain of the PMZ molecule is not metabolized. Demethylation only takes place at the N terminal methyl groups. The +12 PMZ isotopomer that is deuterated at both N terminal methyl groups is used solely as an analytical internal standard for the LC-MS assay. The mass difference between PMZ and the subsequent SIL PMZ forms is three atomic mass units, which is ideal since no ion overlap between any of the PMZ forms is anticipated. Figure 2 shows the mass spectra (obtained on the Alliance LC-MS system) for unlabeled PMZ with a strong molecular ion at m/z 285. The +4 PMZ and +7 PMZ forms will be measured as molecular ions at m/z 288 and m/z 291 respectively, and are expected to have clean strong signals similar to the one depicted for PMZ and +4 PMZ in the related research section (Figure 2).

Treatment: Subjects will receive simultaneously an IM injection (12.5 mg), an oral capsule (12.5 mg) and a rectal suppository (12.5 mg) of PMZ as unlabeled and the two SIL forms of PMZ, respectively. The treatments will be administered on separate days once during each session. All treatments will be administered in the morning to avoid variability due to circadian rhythms. Uniform bed times and meal times will be maintained during the experimental period. The experimental

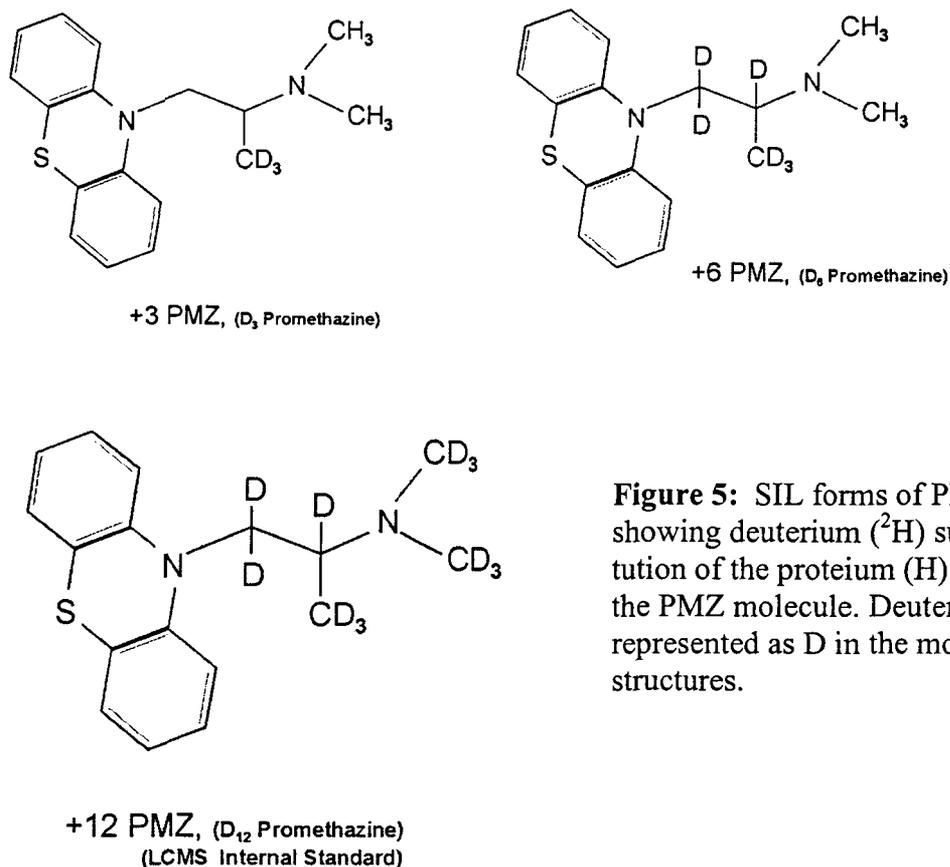


Figure 5: SIL forms of PMZ showing deuterium (²H) substitution of the proterium (H) atoms on the PMZ molecule. Deuterium is represented as D in the molecular structures.

design includes serial blood and saliva sampling, and void-by-void urine collections after administration of PMZ.

Blood, saliva, and urine samples will be collected on experiment days according to the schedule in Table 2. Blood samples (7 mL) will be collected into heparinized vacutainers from an indwelling catheter placed in the antecubital vein of the arm. The volume of blood has been determined, based on the volume required for the determinations of parent drug forms and metabolite concentrations for each session/treatment of each phase. A total of 504 ml of blood will be collected over the entire study period from each subject at four sessions x 18 draws/session x 7 ml/draw; each 72 h sample collection period will require 126 ml. Stimulated mixed saliva samples will be collected using Salivettes® (Sarstedt Inc.). Blood samples will be centrifuged at 3000 rpm, samples transferred to cryotubes and stored frozen at -70°C until analyzed. Urine samples will be collected into labeled polycarbonate bottles, volumes measured, and aliquots (30 ml) will be stored frozen at -70°C for future analysis.

Table 2: Sample / Data Collection Schedule

Sample Type / Test	Collection Times
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Blood and Saliva	0, 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 8, 12, 24, 30, 36, 48, 54, 60 and 72 h post dose
Urine	Pre-dose & PRN voids for 72 h post dose
WinSCAT	Pre-dose & immediately following each blood draw beginning 3 h post dose.
ARES	Pre-dose & immediately following each blood draw.
Sleep Logs	Pre-dose & immediately preceding each blood draw.
Actigraphy	Continuous for 72 h post dose

Sample Analyses. Concentrations of PMZ and metabolites in plasma, saliva, and urine will be determined with a modified liquid chromatography-mass spectrometry (LC-MS) method described in the Preliminary Studies section of this proposal. This method uses an on-line solid-phase extraction coupled with the LC-MS system. The assay involves trapping the analytes onto the extraction column, whereas the urine/plasma matrix are washed out, and column switching valve then turns onto the analytical column, where analytes are eluted in back-flushing mode and resolved within the analytical column. The method allows for automated sample clean up and trace enrichment, as well as provides more reproducible results. Concentrations of PMZ, PMZ sulfoxide, desmethyl PMZ sulfoxide, and desmethyl PMZ will be determined in plasma and urine; while PMZ and PMZ sulfoxide will be determined in saliva. LC-MS analysis of the samples will allow determination of concentrations of the non-isotopic and the two SIL PMZ parent compounds and corresponding metabolites.

Cognitive Performance Testing. WinSCAT is a Windows-based battery of neurocognitive assessment tests that have been validated for different clinical paradigms. Its purpose is to allow researchers and behavioral health care providers a means to objectively evaluate neurocognitive function. It consists of five tests excerpted and modified from the Automated Neurocognitive Assessment Metrics (ANAM) battery of tests developed by the U.S. Navy (Levinson and Reeves, 1994; Reeves et al., 1993). It is a clinical assessment tool designed for repeated measurements. The WinSCAT is a Windows based program and takes about 10-15 minutes to complete the test battery. ARES is a Palm based battery of neurocognitive assessment tests similar to WinSCAT. It implements three tests excerpted and modified from ANAM. The types of functions evaluated by these tests include memory and immediate recall, sustained concentration and attention, verbal working memory, visual working memory and math skills. Subjects will be asked to take the test batteries at least six times during the base line data collection period of the study prior to the initiation of experimentation. They may be asked to attend three 60-minute training sessions before the study begins.

Karolinska Sleepiness Score (KSS). Drowsiness caused by the drug will be measured by subjective sleepiness reports using KSS. Sleep researchers commonly use the KSS to evaluate subjective alertness/sleepiness and this is sensitive to impairments caused by

sleep deprivation and circadian variations. We will use the test results to assess PMZ effects.

d. Data analysis and anticipated conclusions

Pharmacokinetic and Pharmacodynamic Analyses. Concentration vs. time curves will be generated for the unlabeled and SIL PMZ analogs, and their respective metabolite(s) in plasma and urine, these data will be analyzed by standard pharmacokinetic data analysis programs like RSTRIP (MicroMath Scientific, Salt Lake City, UT), WinNonlin (Pharsight Corp., Mountain View, CA) and Boomer (Bourne, 1989), a nonlinear regression analysis program. Absorption and elimination rates will be calculated using noncompartmental parametric estimation methods. Bioavailability will be calculated from the area under the plasma and saliva concentration-time curves (Gibaldi and Perrier, 1982) of the parent compounds using WinNonlin software. Pharmacodynamic effect(s) as a function of drug concentration will be assessed using results from the WinSCAT test scores, actigraphy and KSS data, and physiological variables data. Standard pharmacodynamic analytical methods (Ross, 1990) will be employed to estimate onset, duration and extent of effect, time for minimum and maximum effect, and dose-response relationship of the drug.

Actigraphy data will be reduced using vendor-provided software (Mini Mitter, Inc., Bend, OR) after downloading to a PC via serial port. Sleep and activity parameters (percent daytime activity, sleep latency, wake after sleep onset, duration and efficiency) can be calculated and the effect of PMZ on these parameters evaluated. These objective sleep data will be compared with the data from subjective sleep logs.

WinSCAT is designed to provide an immediate feedback of cognitive performance to the user. An easy to interpret set of scores, "throughput" scores, is automatically created following each session. These scores are a combination of response time and number of correct responses. Higher scores indicate better performance. A table of the throughput scores for all of a subject's sessions can be presented via the SCAT menu. Treatment test scores are compared to the subject's baseline/pre-test scores. Many other data points are also recorded during a session and those data are put into an ASCII text file where they can be exported to databases or statistical analysis programs.

ARES is a software package for the Palm Operating System. It implements a subset of WinSCAT testing. Response time, number of correct responses, and "throughput" scoring are scored similarly to WinSCAT. Compared with WinSCAT, it operates on Palm PDAs and is therefore more portable, and more desirable to use during spaceflight. Additionally, because it is a subset of WinSCAT, it is a shorter test to take.

Statistical Analysis. All data from the two phases will be analyzed separately as well as collectively between sessions to identify effects of microgravity on bioavailability and pharmacokinetics and saliva-to-plasma ratios. Pharmacokinetic parameters, e.g. absorption rate (K_a), bioavailability (F) and biological half-life ($t_{1/2}$) derived from saliva and plasma concentrations-time profiles and ratio of plasma to saliva concentrations over the entire drug disposition period will be assessed using standard regression

methodology. In cases where the non-Gaussian distribution of a response variable precludes standard analysis of variance, a substitute analysis will be made using a generalized linear or nonlinear model with the appropriate distributional family (McCulloch and Nelder, 1989). These data will be also used to determine pharmacodynamic parameters e.g. time for onset of effect, minimum and maximum effect and duration of effect. These data will be further analyzed to determine the concentration-response and dose -response relationship of PMZ.

The level of significance for all statistical tests will be 0.05. Estimates of statistical power for the study indicate that differences between treatment means of 20% can be detected for 8 subjects per treatment with power of >0.9 using analysis of variance on the WinSCAT performance parameters under the implied Gaussian assumption. However to allow for anticipated skewness of the distributions of some responses, we have increased our sample size to 12. Physiological effects of PMZ will be assessed by comparing data collected on all of the bioavailability and pharmacokinetic parameters from all sessions.

Anticipated Conclusions. Results of this study will provide much-needed information on the pharmacokinetics, bioavailability, and pharmacodynamics of PMZ that may have a direct bearing on treatment with this drug for motion sickness during space flights. We expect to prove that the bioavailability of the three formulations of PMZ will be reduced during spaceflight (Hypotheses A and C). We expect that these changes in the pharmacokinetics and bioavailability of PMZ during ABR will be similar to the ones observed with acetaminophen and scopolamine during space flight. We will use these data for developing a pharmacokinetic model for PMZ that can estimate changes in these parameters of drug dynamics during space flight. We expect to validate ABR as a reliable ground-based microgravity analog for bioavailability and pharmacokinetic studies by comparing results of this study to those that will be collected from a similar investigation approved for flight manifestation.

A significant relationship is expected between saliva and plasma concentrations that will be consistent between different doses and routes of administration, but the ratio of saliva to plasma may be different between ambulatory and ABR conditions (Hypothesis B). We expect a strong positive correlation between pharmacokinetic and pharmacodynamic parameters estimated from the plasma and saliva concentration data in both phases of the study. Finally, results of this study will provide a means for establishing noninvasive methods of pharmacodynamic and therapeutic assessment of neuroactive drugs (Hypothesis D).

We expect that results from this study will serve as a basis for methods refinement and modification of an approved (NRA-96-OLMSA-01) flight protocol awaiting manifestation on future flights.

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IX. Human Subjects.

a. Detailed Description of Human Subjects. Twelve healthy, non-smoking subjects between the ages of 21 and 45 years will participate in the study. All subjects will be screened and selected through the subject recruitment facility of the appropriate institution (NASA Johnson Space Center or UTMB GCRC) according to the inclusion and exclusion criteria listed below.

Inclusion criteria:

1. Male or female
2. Age 21-45
3. Normal weight for body size
4. General good health
5. Renal and hepatic function within normal ranges
6. Able to provide written informed consent to participate

Exclusion criteria:

1. Drug allergies, especially to phenothiazine-type agents
2. Use of prescription or over-the-counter medications within one week of starting the study
3. Use of an investigational drug within 30 days of starting the study
4. Tobacco smoking within the past year
5. Blood donation or significant blood loss within 30 days of starting the study
6. Significant gastrointestinal disorder, asthma, or seizure disorders
7. History of alcohol or other drug abuse
8. Pregnancy or suspected pregnancy, or lactation
9. Treatment during menstruation will be avoided
10. Hematocrit values less than 41% for males and 37% for females

Aside from the criteria listed above, no subpopulations or special classes of subjects will be sought or avoided.

b. Sources of Research Material. All material and data will be obtained specifically for research purposes. Samples of blood, saliva, and urine will be obtained from the subjects at specified times or intervals. Data obtained more directly will come from subjects taking the computerized WinSCAT and ARES tests to measure their cognitive performance, wearing an actiwatch that monitors their sleep and activity, and using the Space Therapeutic Assessment Recorder computer software to report on their sleepiness.

c. Potential Risks.

Stable isotope dosing: This study involves the use of non-radioactive forms of promethazine (PMZ) labeled with a stable isotope (deuterium). The use of these labeled forms of PMZ does not involve any greater risk to subjects than that with unlabeled PMZ. Deuterated isotopomers of drugs are used extensively in clinical, pharmaceutical, and biochemical research and have a very good safety record.

Blood sampling: All 4 sessions require multiple blood samples. To avoid repeated venipunctures, we will take samples from an Intra-Cath® indwelling catheter placed in the antecubital vein at the beginning of each session. A separate venipuncture will be needed only for the sample at 72 hours after drug administration. The intracath will be removed at 72 hours to minimize the chance of infection at the injection site. A small risk of infection at the intracath site will remain, as well as a small risk of formation of a blood clot or hematoma associated with this procedure. The likelihood of any of these complications is remote.

The volume of blood drawn from each subject will be about 126 ml over a 72-h period (18 times X 7 ml) and a total of 506 ml over the total period of the experiment (4 sessions over at least six months). A minimum of 7-d wash-out time will separate sessions or treatments. The body can replace 50 to 200 ml of whole blood in a 24-h period. Blood donation centers allow 1 unit (400 to 450 ml) of blood to be drawn every 8 weeks. Blood collection schedules used in this experiment protocol will fall within those guidelines. The total amount of blood withdrawn during one 72-h protocol is about 126 ml, and within one month a maximum of 252 ml, and is within the limits established by the American Association of Blood Banks.

Drug administration: The total dose of PMZ for each session of this study is 37.5 mg. All of these doses are within the therapeutic range and are not expected to cause serious side effects. Common side effects are drowsiness, sedation, impaired psychomotor performance, and movement disorders. These effects are transient. No other known ill effects are expected from drug administration. Intramuscular injection of PMZ may cause some pain and irritation at the site of injection, and use of the suppository form of PMZ may cause rectal irritation.

Adverse reactions and contraindications: Risks common to all drug administrations include allergic reactions and exacerbation of pre-existing conditions. We do not anticipate any serious reactions because this drug (PMZ) has been used clinically for many years.

Side effects of PMZ: Common side effects of PMZ are drowsiness, light-headedness, daytime sedation, dizziness, impaired psychomotor performance, and movement disorder.

d. Recruitment and Consent. Subjects will be recruited through the human subject recruiting facility at either the NASA Johnson Space Center (JSC). If the JSC facilities are used, the study will first be verbally described to each potential subject by an employee of the recruiting facility, and preliminary questions will be asked to determine whether the subject is willing and suitable for this study. Potential subjects will attend a briefing session at which the principal investigator will describe the study in detail and present a written layman's summary and consent form. The subjects will be encouraged to ask questions or to obtain further clarifications of the study purpose, risks, or procedures. Subjects will then be carefully screened for medical risk factors (Air Force Class III physical or equivalent). Once a subject has been medically cleared, a written

informed consent explaining procedures and monitoring will be obtained by the principal investigator. The informed consent document approved by the JSC Committee for the Protection of Human Subjects and the document submitted to the UTMB IRB for approval are attached.

e. Protection Against Risk. A flight surgeon will be on call and available for consultation during the experiment.

Blood sampling: Qualified technicians using appropriate equipment and precautions will carry out blood sampling procedures. Astronauts are trained in blood draw and catheter placement procedures and will perform the catheter placement and venipuncture procedures. The catheter will be fixed in place with a special tape and adhesive combination (Op-Site®). An antiseptic ointment will be applied at the intracath site to reduce the chance of infection. A heparin lock will be maintained to reduce the chance of clot formation or hematoma. Care will be taken to avoid injection of heparin into the vein by using just enough heparin (0.1 ml) to maintain a heparin lock, withdrawing residual volume from the intracath before sampling, and flushing the intracath with saline after each draw.

Drug administration: Subjects will be briefed on PMZ side effects. Astronauts will be trained on the proper administration of the dosage forms. A physician will be on call for the rest of the study period.

Adverse reactions and contraindications: Subjects will be medically screened for any pre-existing conditions and questioned during the pre-test physical about previous drug reactions and allergies. Female subjects are required to take a pregnancy test at the beginning of the study. At least one technician will have knowledge of the drug administered (placebo or PMZ) during each test. This information will be made available to the physician for medical treatment or evaluation of side effects. Intravenous solutions of saline or D5W will be available in the room for emergency use. Injectable diphenhydramine will be kept in the room for emergencies of idiosyncratic and allergic reactions due to drug administration. In the event of an unexpected reaction, a flight trained physician will be on hand for consultation.

Side effects of PMZ: The subjects will be briefed about side effects. They will be warned of the possible sedation effects of the drug.

Protection of confidentiality: Confidentiality of all data will be maintained. Laboratory records will use any subject identifying information; rather, records will be assigned a numerical code, the decryption of which will be maintained by a minimal number of personnel. However, should a life-threatening abnormality be detected during the study, the investigator will notify the subject and the assigned JSC physician. Such information will be used for care and medical follow-up only. Each subject will be assured that his or her identity will not be released to the public without consent, unless specifically required by law.

f. Risks vs. Benefits. All the procedures used in the study are minimally invasive and pose minimal to reasonable risks to the subjects. However, the knowledge to be gained from the research will be important for the health of astronauts. The study will provide information about the dynamics and therapeutic index of promethazine during simulated microgravity conditions. This information will help flight surgeons determine the most appropriate means by which to administer promethazine, as well as help to determine specific physiological alterations induced by microgravity. Because many astronauts take promethazine to prevent space motion sickness, the results of this study will help maintain astronaut health and safety.

g. Data Safety Monitoring Plan.

1) Plan for safety review of data. We estimate the risk level of the study to be low. Vulnerable populations will not be included. The persons responsible for monitoring the safety environment of the participants will be the principal investigator, Lakshmi Putcha, Ph.D.; the medical monitor (physician). A Test Readiness Review Committee will convene before the study begins, to review safety precautions and procedures. After the nurse administers promethazine to a subject, a physician who can be present within 15 min will be on call for the entire study period. Subjects will be released at the end of each study protocol after examination by the nurse and verbal approval of release by the attending physician. The study is expected to last 2 years (4 to 6 months for each subject). All key personnel involved with the study have completed training in the protection of human research participants mandated by the U.S. DHHS Office for Human Research Protections (OHRP).

2) Plan for monitoring. All unanticipated or serious adverse events will be reported immediately to the Johnson Space Center (JSC) Committee for the Protection of Human Subjects (CPHS). An oral report will be given within 24 hours, and a written report within 48 hours of the event.

3) Plan for assessing the degree of severity and attribution of adverse events. The medical monitor or alternate medical monitor of the study will grade any adverse event by the NASA JSC CPHS Adverse Event (AE) Grading Scale. The principal investigator will use the attribution scale (unrelated, possible, probable, or definite) to judge whether an adverse event is associated with a treatment or procedure used in the study.

4) Plan for annual reporting of adverse events. An annual written report of any adverse events will be made to the JSC CPHS.

X. Inclusion of Women and Minorities. Men and women of any race or ethnic background may be selected for the proposed study. All subjects will be screened and selected through the test subject recruitment facility of NASA JSC according to the inclusion and exclusion criteria listed below.

Inclusion criteria:

1. Male or female
2. Age 21-45
3. Normal weight for body size
4. General good health, as determined by an Air Force Class III Flight Physical
5. Renal and hepatic function within normal ranges
6. Able to provide written informed consent to participate

Exclusion criteria:

1. Drug allergies, especially to phenothiazine-type agents
2. Use of prescription or over-the-counter medications within 3 days of starting the study
3. Use of an investigational drug within 30 days of starting the study
4. Tobacco smoking within the past year
5. Blood donation or significant blood loss within 30 days of starting the study
6. Significant gastrointestinal disorder, asthma, or seizure disorders
7. History of alcohol or other drug abuse
8. Pregnancy or suspected pregnancy, or lactation
9. Treatment during menstruation will be avoided
10. Hematocrit values less than 41% for males and 37% for females

Aside from the criteria listed above, no subpopulations or special classes of subjects will be sought or avoided.

Subjects will be enrolled for at least six months each beginning in March 2005.

XI. Inclusion of Children. The research topic is irrelevant to children. The knowledge to be gained from the research will be important for the health of astronauts, all of whom are adults. The study is designed to investigate differences in bioavailability as a function of route of administration and to establish noninvasive methods for assessing pharmacodynamics of promethazine. This information will help flight surgeons determine if astronauts may have side effects after receiving promethazine by one of three different routes of administration. Because many astronauts take promethazine to prevent space motion sickness, the results of this study will help maintain astronaut health and safety. Therefore, the subject population of the study models that of the astronaut corps to address the needs of this very specific population.

XII. Use of the General Clinical Research Center.

a. Laboratory investigations.

1. Investigators' laboratories. All analyses of samples and of data from the actigraphs and WinSCAT test battery will be performed in the investigators' laboratories. Concentrations of promethazine (PMZ) and its metabolites in blood plasma, saliva, and urine will be determined with a modified liquid chromatographic-mass spectrometry (LC-

MS) method. This method uses an on-line solid-phase extraction coupled with the LC-MS system. Concentrations of PMZ, PMZ sulfoxide, desmethyl PMZ sulfoxide, and desmethyl PMZ will be determined in plasma and urine; PMZ and PMZ sulfoxide will be determined in saliva. Concentration vs. time curves will be generated for the unlabeled and stable-isotope-labeled PMZ analogs, and their respective metabolite(s) in plasma and urine, and these data will be analyzed by exponential stripping and parametric estimations using standard pharmacokinetic data analysis programs. Absorption and elimination rates will be calculated using noncompartmental parametric estimation methods. Bioavailability will be calculated from the area under the plasma and saliva concentration-time curves of the parent compounds using WinNonlin software. Sleep and activity variables (percent daytime activity, sleep latency, wake after sleep onset, duration and efficiency) will be calculated from actigraph data, and the effect of PMZ on these variables will be evaluated. These objective sleep data will be compared with the data from subjective sleep logs (Karolinska Sleepiness Score, KSS). After each WinSCAT and ARES session, an easy-to-interpret set of scores, "throughput" scores, is automatically created. These scores are a combination of response time and number of correct responses. Higher scores indicate better performance. A table of the throughput scores for all of a subject's sessions can be presented via the SCAT or ARES menu. Treatment test scores are compared to the subject's baseline/pre-test scores. Pharmacodynamic effect(s) as a function of drug concentration will be assessed using results from the WinSCAT and ARES test scores, actigraphy, KSS data, and physiological data. Standard pharmacodynamic analytical methods will be used to estimate onset, duration, and extent of effect, time for minimum and maximum effect, and dose-response relationship of the drug.

2. Hospital laboratories. Not anticipated.
3. Outside laboratories. Not anticipated.

XII. Grant or contract support. Please see the attached form.